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Research Article

# Acute Oral Toxicity and Tolerance of Emamectin Benzoate, Profenofos, and a Sulphur-Based Product in Broiler Chickens

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**ABSTRACT**

**Introduction:** Pesticide residues in feed and water can adversely affect broiler chicken production. This preliminary study aimed to determine the maximum tolerated dose of emamectin benzoate (EB), profenofos, and a sulphur-based product, estimate the profenofos $LD_{50}$, and evaluate the efficacy of dietary charcoal plus L-carnitine in mitigating the resulting toxicity in broiler chickens.

**Materials and methods:** A total of 240 broiler chickens of both sexes were procured at 10 days of age and divided into a control and three treatment groups. The EB was given in drinking water at 300, 500, 1800, and 2300 mg/kg of body weight (BW), profenofos was administered by oral gavage in eight independent dose groups (2.55, 5, 7.5, 10, 20, 50, 100, and 600 mg/kg BW) for acute lethality; and the sulphur-based product was administered in feed (300-2300 mg/kg). The control group was sampled on day 15. Chickens received the EB and sulphur-based product on days 17 and 19, respectively, and the blood samples were collected on days 18 and 20 from the groups. Serum glutamate pyruvate transaminase (SGPT), serum glutamate oxaloacetate transaminase (SGOT), alkaline phosphatase (ALP), creatinine, alongside mortality and clinical signs, were recorded over 7 days. Dietary charcoal plus L-carnitine was then given to surviving broiler chickens in the EB, profenofos, and sulphur-based product groups.

**Results:** Profenofos induced dose-dependent mortality, with an acute oral $LD_{50}$ of approximately 4.7 mg/kg BW. In contrast, EB produced no mortality or clinical signs up to 2300 mg/kg. Across all four EB groups, ALP levels were elevated compared to the control group, whereas SGPT, SGOT, and serum creatinine remained largely unchanged. However, because chickens in EB groups were sampled at 18-20 days of age, the observed ALP elevation could not be distinguished from age-related physiological changes. In contrast, the sulphur-based product caused no mortality or clinical signs at any dose tested. Charcoal plus L-carnitine did not prevent feed refusal in profenofos survivors.

**Conclusion:** The current findings provided an acute oral profenofos $LD_{50}$ of 4.7 mg/kg BW in broiler chickens and tolerance observations for EB and a sulphur-based product up to 2300 mg/kg BW.

## 1. Introduction

In modern crop production, farmers use pesticides extensively to control insect pests, weeds, and fungal diseases, thereby protecting crop yield[1]. Although this excessive pesticide use supports agricultural productivity and food security at the farm level, the same compounds can reach and harm non-target species, including farmed livestock such as poultry[2,3]. Broiler chickens, integral to the global food supply chain, are particularly vulnerable to pesticide exposure[2,3]. This exposure primarily occurs through contaminated feed and water, leading to potential health risks and economic losses[4]. Such contamination can arise at any stage of cultivation, processing, or storage. A range of pesticide-residue classes have been detected in poultry feed in India, including organophosphates,

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carbamates, pyrethroids, organochlorines, and herbicides[5,6]. Organophosphates such as chlorpyrifos, diazinon, and malathion are neurotoxic and can adversely affect both animals and humans[7]. Carbamates such as carbaryl and methomyl inhibit cholinesterase enzymes and have been shown to be immunotoxic in chickens[8]. Synthetic pyrethroids such as cypermethrin, deltamethrin, and permethrin mimic natural insecticidal compounds[6]. Organochlorines such as dichlorodiphenyltrichloroethane (DDT) and hexachlorocyclohexane (HCH), although largely banned, may still persist as residues[9]. In India, although the Food Safety and Standards Authority of India set permissible pesticide-residue limits for feed, weak enforcement and limited monitoring remain significant barriers to effective control[10].

Despite poultry's critical role in agriculture, comprehensive data on the toxicological effects of commonly used pesticides on broiler chickens are lacking. Most toxicity studies have focused on mammals, such as rats, due to their widespread use in laboratory investigations[11]. However, the physiological and metabolic differences between mammals and poultry necessitate species-specific toxicity assessments to ensure accurate risk evaluation and safe pesticide use in poultry farming[12].

Emamectin benzoate is a semi-synthetic avermectin insecticide that acts mainly on glutamate-gated chloride channels and can also affect gamma-aminobutyric acid-mediated neurotransmission in susceptible animals[13]. In non-target avian species such as mallard duck and northern bobwhite quail, reported toxic effects included lethargy, reduced feed intake, and dose-related mortality, although susceptibility differs widely among species[7]. Profenofos is an organophosphate insecticide that inhibits acetylcholinesterase, leading to acetylcholine accumulation and subsequent neurotoxicity[14]. In poultry, profenofos exposure has been associated with altered biochemical markers such as transaminases and alkaline phosphatase, indicating hepatic stress and metabolic disturbance[1]. Sulphur is one of the oldest and most widely used agricultural fungicides and acaricides, valued for its broad activity against fungi and mites and its low mammalian and avian toxicity[15]. Because sulphur-based products are applied extensively in crop protection and can enter the poultry feed chain, and because their acute oral tolerance in broiler chickens at high doses has not been well characterized, a commercial sulphur product was included in the present study[5,15].

Emamectin benzoate and profenofos presented potential health hazards to non-target organisms, including broiler chickens. Previous poultry studies demonstrated that pesticide exposure can produce species-specific toxic effects that differ in severity and target-organ response[6,12]. Emamectin benzoate has demonstrated acute toxicity in some avian species[16]; profenofos has been associated with significant biochemical disturbances in chickens[17,18], while sulphur was generally regarded as having low oral toxicity[15]. The acute oral tolerance of emamectin benzoate, profenofos, and a sulphur-based product has not been systematically characterized in broiler chickens.

Activated charcoal and L-carnitine have both been investigated as feed additives in broiler chickens[2,6,19]. Activated charcoal has a large adsorptive surface area and has been used to bind dietary toxins and reduce their absorption from the gastrointestinal tract in poultry and other livestock[6]. L-carnitine, a cofactor required for mitochondrial fatty-acid transport and energy metabolism, has been reported to improve growth performance, feed efficiency, and stress tolerance in broiler chickens[2,19]. Accordingly, the present study aimed to determine the maximum tolerated dose (MTD) of emamectin benzoate, profenofos, and a sulphur-based product in broiler chickens, estimate the median lethal dose ($LD_{50}$) of profenofos in broiler chickens, and evaluate the efficacy of dietary charcoal plus L-carnitine in mitigating the resulting toxicity.

## 2. Materials and Methods

### *2.1. Ethical approval*

All experimental procedures involving animals were conducted after receiving approval from the Institutional Animal Ethics Committee (IAEC), Faculty of Veterinary and Animal Sciences, West Bengal University of Animal and Fishery Sciences, Kolkata, India (approval no. 763/GO/Re-S/ReRc-L/03/CCSEA/82/2024-25, dated 12 February 2025). The present study followed the committee's ethical guidelines for animal research and the guidelines of the Committee for the Control and Supervision of Experiments on Animals (CCSEA), Government of India. The study was conducted in Hyderabad, India, and included a 7-day post-dosing observation period for each treatment.

### *2.2. Materials and chemicals*

The following commercial formulations, including emamectin benzoate (5% w/w, JAWS, Ravi Corp Science, Jodhpur, India), profenofos (50% w/w, JASHIN, Rallis India Ltd., Mumbai, India), and a sulphur-based product (80% w/w, THIONUTRI, India) were used. All doses were expressed as milligrams of formulated product per kilogram of body weight (BW). L-carnitine (pure grade; Sarshika Pharmachem LLP, Bangalore, India) and locally procured charcoal (Sagar Charcoal and Firewood Depot, Hyderabad, India) were used for the mitigation experiment.

### *2.3. Animals*

A total of 240 Vencobb broiler chickens of both sexes were procured at 10 days of age from Leos Farm, Kushaiguda, Hyderabad, India, with a mean BW of 528.7 g. Chickens were reared under established management on a commercial broiler starter crumb feed (GT11, Japfa Comfeed India Pvt. Ltd., India). The feed contained 21% crude protein and 3000 kcal/kg metabolizable energy, with 13% moisture and 5% crude fiber, 0.6-1.2% calcium, 0.4-1.2% phosphorus, and 1.2% methionine plus cystine and 0.85% threonine, and was formulated from corn, tapioca, rice bran, soybean meal, meat meal, and a vitamin-mineral

premix. Chickens were housed in groups of five per pen for the emamectin benzoate, sulphur-based product, and control groups, and two per pen for the profenofos groups. Feed and clean drinking water were provided *ad libitum*, except during controlled pesticide administration. Each chicken was weighed immediately before dosing, and its BW was used to calculate the administered dose, so that all doses were expressed per unit BW[20].

### *2.4. Experimental design*

The acute toxic effects of emamectin benzoate, profenofos, and sulphur-based product were evaluated in broiler chickens allocated to a control group and three pesticide-treated groups. A single control group, sampled at 15 days of age, was used as a shared baseline rather than collecting separate age-matched controls at each sampling age. Using a single shared control was a deliberate choice to minimize the total number of chickens used, consistent with the minimal-animal-number approach applied in the profenofos groups. Emamectin benzoate was administered through drinking water, profenofos by direct oral gavage, and sulphur-based product through feed (Table 1). Each pesticide was administered via the route most relevant to real-world exposure, so the three groups were analyzed separately, and no direct potency comparisons were made among them. For the drinking-water (emamectin benzoate) and feed (sulphur-based product) routes, doses were calculated from individual BWs and offered as measured quantities of medicated water or feed; *ad libitum* access was restored once this quantity had been consumed. Because both routes (through drinking-water and feeding) dosed chickens collectively rather than individually, the dose was an estimate based on each chicken's body weight and given to the entire pen. The actual amount ingested by any single chicken was not independently verified and may have differed from this nominal value. Additionally, the actual concentrations in the prepared water or feed were not recorded and could not be reconstructed, meaning the reported doses were nominal target values rather than verified concentrations. This pen-level dosing limitation did not apply to profenofos, which was administered to each chicken individually by oral gavage. Separate groups of broiler chickens were assigned to each dose level, and no chickens received more than one dose. Group sizes differed because the groups addressed different objective. The emamectin benzoate, sulphur-based product, and control groups were used to detect sub-lethal changes in continuous biochemical variables, which required adequate replication, and each therefore comprised five broiler chickens of each sex at each sampling age. Using five broiler chickens per group was consistent with previous small-scale toxicity studies in chickens[7,18]. The profenofos groups were designed to evaluate acute lethality, a quantal endpoint. To adhere to international guidelines promoting sequential testing with minimal animal use per stage, this group used a sequential design and did not stratify groups by sex.

#### *2.4.1. Emamectin benzoate and sulphur-based product*

Emamectin benzoate was assessed in four independent dose groups (300, 500, 1800, and 2300 mg/kg BW) and sulphur-based product in six independent dose groups (300, 600, 900, 1300, 1800, and 2300 mg/kg BW), with serum samples collected for biochemical analysis from each group at 18 and 20 days of age (24 hours after dosing at 17 and 19 days, respectively). Emamectin benzoate and sulphur were tested at doses ranging from sub-lethal up to 2300 mg/kg BW. This maximum exceeds the 2000 mg/kg limit dose specified for avian acute oral toxicity testing[20]. For Emamectin benzoate comparisons, chickens from the control group were sampled at 15 days of age, whereas treated chickens were sampled at 18 and 20 days of age; because serum ALP and creatinine change with normal growth over this interval, differences from the control could be attributed to treatment.

#### *2.4.2. Profenofos*

Chickens were procured at 10 days of age and acclimatized for five days before the start of the study at 15 days of age, consistent with the acclimatization period allowed prior to single-dose oral profenofos administration in broiler chickens by Kafle et al.[17]. Profenofos was administered at 15 days of age, and the resulting $LD_{50}$ was therefore applied to 15-day-old broiler chickens. The selected age range corresponded to the broiler starter phase, defined by the Bureau of Indian Standards as the initial three weeks post-hatch, consisting of pre-starter (days 1-7) and starter (days 8-21) feeding periods[21]. During the starter-feed period (8-21 days), chickens received starter rations and were in an active growth phase, considered the period of greatest relative exposure to any pesticide residue in feed or drinking water. Profenofos was assessed in eight independent groups at 2.55, 5, 7.5, 10, 20, 50, 100, and 600 mg/kg BW to assess acute lethality, administered by oral gavage as 1 mL per chicken. This graded-dose design, in which independent groups each received a single dose level, followed the standard approach for avian acute oral toxicity testing[20]. Profenofos, which caused rapid mortality at the initially tested doses, was reduced to 2.55 mg/kg BW to lower the $LD_{50}$. The control group received the conventional diet and water without pesticide. Across all exposure groups, clinical signs, feed intake, water intake, and mortality were recorded daily throughout the 7-day post-dosing observation period. Group sizes in the profenofos groups were kept to the minimum and increased only when the mortality response was ambiguous. At 5 mg/kg BW, where partial mortality occurred, the group was extended to ten chickens so the $LD_{50}$ could be defined precisely. No statistical comparison was made between profenofos dose groups; moreover, the Spearman-Kärber method employed for $LD_{50}$ estimation was robust to unequal group sizes[22].

**Table 1.** Experimental design for determination of the maximum tolerated dose of emamectin benzoate, profenofos, and sulphur-based product in broiler chickens

| Groups | Treatment dose (mg/kg of body weight) | Route | Sex | Number of chickens per sex per sampling age | Total (number) |
|---|---|---|---|---|---|
| Control | None | - | Male and Female | 5 | 10 |
| EB1 | 300 mg EB/kg BW | Drinking water | Male and Female | 5 | 20 |
| EB2 | 500 mg EB/kg BW | Drinking water | Male and Female | 5 | 20 |
| EB3 | 1800 mg EB/kg BW | Drinking water | Male and Female | 5 | 20 |
| EB4 | 2300 mg EB/kg BW | Drinking water | Male and Female | 5 | 20 |
| PF1 | 2.55 mg PF/kg BW | Oral gavage | Male and Female (not stratified) | - | 2 |
| PF2 | 5 mg PF/kg BW | Oral gavage | Male and Female (not stratified) | - | 10* |
| PF3 | 7.5 mg PF/kg BW | Oral gavage | Male and Female (not stratified) | - | 2 |
| PF4 | 10 mg PF/kg BW | Oral gavage | Male and Female (not stratified) | - | 4 |
| PF5 | 20 mg PF/kg BW | Oral gavage | Male and Female (not stratified) | - | 2 |
| PF6 | 50 mg PF/kg BW | Oral gavage | Male and Female (not stratified) | - | 2 |
| PF7 | 100 mg PF/kg BW | Oral gavage | Male and Female (not stratified) | - | 2 |
| PF8 | 600 mg PF/kg BW | Oral gavage | Male and Female (not stratified) | - | 4 |
| S1 | 300 mg sulphur/kg BW | Feed | Male and Female | 5 | 20 |
| S2 | 600 mg sulphur/kg BW | Feed | Male and Female | 5 | 20 |
| S3 | 900 mg sulphur/kg BW | Feed | Male and Female | 5 | 20 |
| S4 | 1300 mg sulphur/kg BW | Feed | Male and Female | 5 | 20 |
| S5 | 1800 mg sulphur/kg BW | Feed | Male and Female | 5 | 20 |
| S6 | 2300 mg sulphur/kg BW | Feed | Male and Female | 5 | 20 |
| **Total allocated chickens** | | | | | 238 |

EB: Emamectin benzoate, PF: Profenofos, S: Sulphur-based product, BW: Body weight. Separate groups of broiler chickens were assigned to each dose level, and no chickens received more than one dose. Doses shown for the emamectin benzoate and sulphur-based groups were nominal values calculated from individual body weight and pooled per pen. The profenofos lethality assessment was not stratified by sex or age. A single control group (sampled at 15 days of age) served as a shared baseline for all treatment groups. *Group sizes in the profenofos group were minimal by design and were increased only at 5 mg/kg of body weight, where partial mortality occurred, to define the $LD_{50}$ more precisely.

### *2.5. Serum biochemical analysis*

Approximately 1-2 mL of blood was collected from the wing vein of each chicken 24 hours after exposure using sterile syringes and transferred to clot activator tubes for serum separation. Serum was obtained by centrifugation and used to estimate serum glutamate pyruvate transaminase (SGPT) and serum glutamate oxaloacetate transaminase (SGOT) using the colorimetric method of Reitman and Frankel[23]. Alkaline phosphatase (ALP) was evaluated by the p-nitrophenyl phosphate method, and serum creatinine by the modified Jaffe (alkaline picrate) kinetic method, using commercial diagnostic kits (Tulip Diagnostics [P] Ltd., India) on a semi-automatic biochemistry analyzer (INNOLAB 200, India) at Medicity Diagnostic Center, Nagole, Hyderabad, India. Serum uric acid, phosphorus, and blood cholinesterase activity were not evaluated.

### *2.6. Median lethal dose determination*

The $LD_{50}$ was determined for profenofos only, because emamectin benzoate and sulphur-based products produced no mortality at any tested dose, whereas profenofos caused dose-related death. Graded oral doses of profenofos were administered, and mortality was recorded over the 7-day post-dosing observation period. Because mortality increased sharply from 0% at 2.55 mg/kg to 100% at ≥ 7.5 mg/kg, the $LD_{50}$ was estimated by the Spearman-Kärber method[20], which is appropriate for steep quantal dose-response data.

### *2.7. Charcoal and L-carnitine*

The MTD of each pesticide, defined as the highest dose that caused no mortality during the 7-day observation period, was identified from the graded-dose mortality data and used in the present experiment. A total of 20 broiler chickens that survived emamectin benzoate exposure and the 20 that survived sulphur-based product exposure were each divided into five subgroups of four chickens and received a single combined charcoal and L-carnitine supplement incorporated into the feed at one of four inclusion levels (3, 5, 10, or 20 g per chicken), and the last group received the standard diet without the additive as a control, over the 7-day observation period. Only seven broiler chickens survived profenofos exposure, two at 2.55 mg/kg and five at 5 mg/kg BW, which was too few to subdivide, so these were kept as a single group (Table 2). These levels were chosen as an empirical ascending range to test for a dose-dependent protective effect, based on the established adsorptive capacity of charcoal for dietary toxins[6] and the role of L-carnitine in energy metabolism and stress tolerance in broiler chickens[2,19]. The broiler chickens were monitored daily for 7 days after pesticide administration for feed intake, feed refusal, and general clinical condition. To evaluate additive efficacy, feed refusal following pesticide administration was employed as the outcome metric.

**Table 2.** Dietary charcoal and L-carnitine inclusion levels in broiler chickens exposed to emamectin benzoate, profenofos, and a Sulphur-based product over a seven-day post-dosing period

| Pesticide groups | Pesticide dose (mg/kg of body weight) | Charcoal + LC level (g/bird) | Chickens (number) | Outcome over 7 days |
|---|---|---|---|---|
| Emamectin benzoate | Diet without pesticide | 0 (control group) | 4 | No feed refusal or mortality |
| | MTD of 2300 mg/kg BW | 3 | 4 | No feed refusal or mortality |
| | | 5 | 4 | No feed refusal or mortality |
| | | 10 | 4 | No feed refusal or mortality |
| | | 20 | 4 | No feed refusal or mortality |
| Profenofos | 2.55 and 5 mg/kg BW | Not sub-grouped | 7 | Feed refusal not prevented |
| Sulphur-based product | Diet without pesticide | 0 (control) | 4 | No feed refusal or mortality |
| | MTD of 2300 mg/kg BW | 3 | 4 | No feed refusal or mortality |
| | | 5 | 4 | No feed refusal or mortality |
| | | 10 | 4 | No feed refusal or mortality |
| | | 20 | 4 | No feed refusal or mortality |
| **Total number of chickens used** | | | 47 | |

LC: L-carnitine, MTD: Maximum tolerated dose, 2300 mg/kg BW for emamectin benzoate and Sulphur-based product, BW: Body weight. Because no feed refusal or mortality was observed with either pesticide at any inclusion level (including the additive-free control subgroup), it was not possible to evaluate the effectiveness of charcoal plus L-carnitine in the emamectin benzoate or sulfur-based product groups. Charcoal and LC were administered through feeding in all groups.

### *2.8. Statistical analysis*

Data were expressed as mean ± SEM (n = 5 per group). Statistical analysis was performed using R software Version 4.5.2 (R Foundation for Statistical Computing, Vienna, Austria). For each biochemical parameter, and separately for each sex, treatment groups were compared by one-way analysis of variance (ANOVA); where the ANOVA was significant, each treated group was compared with the corresponding control using unpaired t-tests with Holm's correction for multiple comparisons. A p-value less than 0.05 was considered significant.

## 3. Results

### *3.1. Emamectin benzoate*

#### *3.1.1. Emamectin benzoate at 300 mg/kg of body weight*

In broiler chickens administered emamectin benzoate at 300 mg/kg BW, no significant changes in SGPT, SGOT, or serum creatinine were observed in either sex at either sampling age (18 and 20 days) compared to the control group ($p > 0.05$). This result indicated that these parameters remained stable during the observation period (Table 3). In contrast, in broiler chickens administered emamectin benzoate at 300 mg/kg BW, ALP increased significantly at 18 and 20 days of age, compared to the control group, in both sexes ($p < 0.05$). In male broiler chickens, ALP significantly increased from 2134 ± 245.04 IU/L in the control to 5091 ± 306.27 IU/L at 18 days and 17090 ± 854.64 IU/L at 20 days. In female broiler chickens, ALP

significantly increased from 2084 ± 195.11 IU/L in the control to 5324 ± 322.54 IU/L at 18 days and 9690 ± 687.23 IU/L at 20 days. The control was sampled at 15 days of age, whereas treated chickens were sampled at 18 and 20 days; because serum ALP and creatinine change with normal growth over this interval, differences from control cannot be attributed to treatment. No clinical signs and no mortality were observed in any group during the study period.

**Table 3.** Serum biochemical parameters in broiler chickens administered emamectin benzoate at 300 mg/kg of body weight, 24 hours after exposure during a 7-day observation period

| Sex | Age | SGPT (IU/L) | SGOT (IU/L) | ALP (IU/L) | Serum creatinine (mg/dL) |
|---|---|---|---|---|---|
| Male | 15 days (control) | 222.2 ± 18.36 | 39.4 ± 3.04 | 2134 ± 245.04[a] | 0.412 ± 0.056 |
| | 18 days (treated) | 165.0 ± 8.28 | 34.0 ± 2.52 | 5091 ± 306.27[b] | 0.320 ± 0.029 |
| | 20 days (treated) | 189.0 ± 14.45 | 30.0 ± 2.93 | 17090 ± 854.64[c] | 0.410 ± 0.034 |
| Female | 15 days (control) | 216.9 ± 15.45 | 38.6 ± 3.19 | 2084 ± 195.11[a] | 0.406 ± 0.044 |
| | 18 days (treated) | 173.0 ± 12.3 | 31.0 ± 2.89 | 5324 ± 322.54[b] | 0.410 ± 0.038 |
| | 20 days (treated) | 169.0 ± 11.21 | 36.0 ± 2.21 | 9690 ± 687.23[c] | 0.620 ± 0.054 |

SGPT: Serum glutamate pyruvate transaminase, SGOT: Serum glutamate oxaloacetate transaminase, ALP: Alkaline phosphatase. [a,b,c] Mean different superscript letters denote significant differences in ALP concentration among the three age points within each column, analyzed separately by sex ($p < 0.05$). SGPT, SGOT, and serum creatinine did not differ significantly. Data are presented as mean ± SEM.

### *3.1.2. Emamectin benzoate at 500 mg/kg of body weight*

In broiler chickens administered emamectin benzoate at 500 mg/kg BW, no significant changes in SGPT, SGOT, or serum creatinine were observed in either sex at either sampling age (18 and 20 days) compared to the control group ($p > 0.05$). These findings indicated that SGPT, SGOT, or serum creatinine remained stable (Table 4). However, ALP increased significantly compared to the control group at 20 days of age in both sexes ($p < 0.05$), reaching 8910 ± 763.24 IU/L in male and 11690 ± 785.33 IU/L in female broiler chickens. The ALP increased to 3691 ± 321.66 IU/L in males and 2984 ± 282.54 IU/L in females at 18 days of age, with no statistically significant difference ($p > 0.05$). When comparing the 300 and 500 mg/kg emamectin benzoate dose levels, the ALP response at 20 days differed by sex. In male broiler chickens the increase in ALP level was greater at 300 mg/kg (17090 ± 854.64 IU/L) than at 500 mg/kg (8910 ± 763.24 IU/L), while in female broiler chickens ALP level was greater at 500 mg/kg (11690 ± 785.33 IU/L) than at 300 mg/kg (9690 ± 687.23 IU/L). This inconsistent, non-monotonic pattern across dose levels was not consistent with a dose-dependent toxic effect and further supported the interpretation that the ALP differences reflected normal age-related variation rather than emamectin benzoate toxicity. No clinical signs and no mortality were observed in any group during the study period.

**Table 4.** Serum biochemical parameters in broiler chickens administered emamectin benzoate at 500 mg/kg of body weight, 24 hours after exposure during a 7-day observation period

| Sex | Age | SGPT (IU/L) | SGOT (IU/L) | ALP (IU/L) | Serum creatinine (mg/dL) |
|---|---|---|---|---|---|
| Male | 15 days (control) | 222.2 ± 18.36 | 39.4 ± 3.04 | 2134 ± 245.04[a] | 0.412 ± 0.056 |
| | 18 days (treated) | 189.1 ± 6.72 | 36.0 ± 3.21 | 3691 ± 321.66[a] | 0.380 ± 0.031 |
| | 20 days (treated) | 200.0 ± 8.44 | 38.0 ± 3.45 | 8910 ± 763.24[b] | 0.390 ± 0.048 |
| Female | 15 days (control) | 216.9 ± 15.45 | 38.6 ± 3.19 | 2084 ± 195.11[a] | 0.406 ± 0.044 |
| | 18 days (treated) | 176.0 ± 10.15 | 32.0 ± 2.78 | 2984 ± 282.54[a] | 0.280 ± 0.032 |
| | 20 days (treated) | 191.0 ± 12.22 | 34.0 ± 4.35 | 11690 ± 785.33[b] | 0.360 ± 0.031 |

SGPT: Serum glutamate pyruvate transaminase, SGOT: Serum glutamate oxaloacetate transaminase, ALP: Alkaline phosphatase. [a,b] Mean different superscript letters denote significant differences in ALP concentrations among the three age points within each column, analyzed separately by sex ($p < 0.05$). SGPT, SGOT, and serum creatinine did not differ significantly. Data are presented as mean ± SEM.

### *3.1.3. Emamectin benzoate at 1800 mg/kg of body weight*

In broiler chickens administered emamectin benzoate at 1800 mg/kg BW, no significant changes in SGPT or SGOT were observed in either sex at either sampling age (18 and 20 days) compared to the control group ($p > 0.05$; Table 5). Mean serum creatinine was numerically higher in the male treated groups at 18 days (0.57 ± 0.069 mg/dL) and 20 days of age (0.61 ± 0.083 mg/dL). At 20 days of age, mean serum creatinine was higher in female broiler chickens (0.54 ± 0.048 mg/dL) than in the control group (0.412 ± 0.056 mg/dL). In males, the mean value was 0.406 ± 0.044 mg/dL. However, none of these differences were statistically significant ($p > 0.05$). The ALP increased significantly compared to the control at 20 days of age in both sexes ($p < 0.05$), reaching 14801 ± 792.22 IU/L in male and 6770 ± 587.23 IU/L in female broiler chickens, whereas the increases at 18 days were not statistically significant compared to the control group ($p > 0.05$).

**Table 5.** Serum biochemical parameters in broiler chickens administered emamectin benzoate at 1800 mg/kg of body weight, 24 hours after exposure during a 7-day observation period

| Sex | Age | SGPT (IU/L) | SGOT (IU/L) | ALP (IU/L) | Serum creatinine (mg/dL) |
|---|---|---|---|---|---|
| Male | 15 days (control) | 222.2 ± 18.36 | 39.4 ± 3.04 | 2134 ± 245.04[a] | 0.412 ± 0.056 |
| | 18 days (treated) | 158.0 ± 7.92 | 32.0 ± 3.57 | 3091 ± 311.05[a] | 0.570 ± 0.069 |
| | 20 days (treated) | 144.0 ± 8.23 | 32.0 ± 2.88 | 14801 ± 792.22[b] | 0.610 ± 0.083 |
| Female | 15 days (control) | 216.9 ± 15.45 | 38.6 ± 3.19 | 2084 ± 195.11[a] | 0.406 ± 0.044 |
| | 18 days (treated) | 206.0 ± 14.25 | 45.0 ± 4.89 | 2698 ± 212.66[a] | 0.290 ± 0.022 |
| | 20 days (treated) | 184.0 ± 10.35 | 37.0 ± 3.91 | 6770 ± 587.23[b] | 0.540 ± 0.048 |

SGPT: Serum glutamate pyruvate transaminase, SGOT: Serum glutamate oxaloacetate transaminase, ALP: Alkaline phosphatase. [a,b] Mean different superscript letters denote significant differences in ALP concentrations among the three age points within each column, analyzed separately by sex ($p < 0.05$). SGPT, SGOT, and serum creatinine did not differ significantly. No clinical signs and no mortality were observed in any group during the study period. Data are presented as mean ± SEM.

### *3.1.4. Emamectin benzoate at 2300 mg/kg of body weight*

In broiler chickens administered emamectin benzoate at 2300 mg/kg BW, no significant changes in SGPT or SGOT were observed in either sex at either sampling age (18 and 20 days) relative to the control group ($p > 0.05$; Table 6). Serum creatinine did not differ significantly from controls in males at any time point or in females at 18 days of age ($p > 0.05$). However, at 20 days, female broiler chickens had a significantly higher mean creatinine level (0.71 ± 0.062 mg/dL) than their female controls (0.406 ± 0.044 mg/dL; $p < 0.05$). The ALP increased significantly relative to the control at 20 days of age in both sexes ($p < 0.05$), reaching 16600 ± 952.58 IU/L in male and 17965 ± 1005.21 IU/L in female broiler chickens, whereas the increases at 18 days were not statistically significant ($p > 0.05$). No clinical signs and no mortality were observed in any group during the study period.

**Table 6.** Serum biochemical parameters in broiler chickens administered emamectin benzoate at 2300 mg/kg of body weight, 24 hours after exposure during a 7-day observation period

| Sex | Age | SGPT (IU/L) | SGOT (IU/L) | ALP (IU/L) | Serum creatinine (mg/dL) |
|---|---|---|---|---|---|
| Male | 15 days (control) | 222.2 ± 18.36 | 39.4 ± 3.04 | 2134 ± 245.04[a] | 0.412 ± 0.056[a] |
| | 18 days (treated) | 171.0 ± 8.35 | 33.0 ± 3.13 | 4120 ± 295.65[a] | 0.330 ± 0.035[a] |
| | 20 days (treated) | 165.0 ± 7.63 | 33.0 ± 2.75 | 16600 ± 952.58[b] | 0.410 ± 0.032[a] |
| Female | 15 days (control) | 216.9 ± 15.45 | 38.6 ± 3.19 | 2084 ± 195.11[a] | 0.406 ± 0.044[a] |
| | 18 days (treated) | 145.0 ± 7.89 | 27.0 ± 1.59 | 4010 ± 246.23[a] | 0.300 ± 0.029[a] |
| | 20 days (treated) | 141.0 ± 8.22 | 42.0 ± 4.86 | 17965 ± 1005.21[b] | 0.710 ± 0.062[b] |

SGPT: Serum glutamate pyruvate transaminase, SGOT: Serum glutamate oxaloacetate transaminase, ALP: Alkaline phosphatase. [a,b] Mean superscript letters compare the three age points within a column, separately for each sex, and differ significantly ($p < 0.05$). SGPT, SGOT, and male serum creatinine did not differ significantly. Data are presented as mean ± SEM.

### *3.2. Profenofos*

At the three highest doses of profenofos, all broiler chickens died within ten minutes of administration; four chickens at 600 mg/kg, and two at each of 100 and 50 mg/kg. Mortality was also complete at the intermediate doses, but occurred more slowly. Both chickens at 20 mg/kg died within one hour, all four at 10 mg/kg within two hours, and at 7.5 mg/kg within a few hours of administration. At 5 mg/kg BW, only some chickens died, and death occurred over a wider time range. An initial pair of broiler chickens was tested, and one died the following day; the experiment was then extended with eight additional chickens housed as two per pen across four pens, and one chicken in each pen died. In total, 5 of the 10 broiler chickens tested at 5 mg/kg died (50% mortality), some within hours and others by the following day. At the lowest dose of profenofos (2.55 mg/kg BW), no mortality was observed in either chicken during the 7-day observation period. Table 7 summarises the dose-related mortality response. Mortality increased sharply from 0% at 2.55 mg/kg to 50% at 5 mg/kg and 100% at 7.5 mg/kg BW and higher doses. Therefore, the $LD_{50}$ of profenofos in broiler chickens was estimated by the Spearman-Kärber method at approximately 4.7 mg/kg BW (95% confidence interval: 3.9-5.6 mg/kg). Since partial mortality occurred only at the 5 mg/kg dose, this interval was considered preliminary. This value was consistent with the low oral $LD_{50}$ previously reported for profenofos in broiler chickens (1.6 mg/kg)[24], indicating that broiler chickens were highly susceptible to profenofos.

**Table 7.** Mortality rate of broiler chickens following single oral administration of profenofos at graded doses over a 7-day observation period

| Profenofos dose (mg/kg of BW) | Broiler chickens (number) | Dead chickens (number) | Mortality (%) | Time to death |
|---|---|---|---|---|
| 600 | 4 | 4 | 100 | Within 10 minutes |
| 100 | 2 | 2 | 100 | Within 10 minutes |
| 50 | 2 | 2 | 100 | Within 10 minutes |
| 20 | 2 | 2 | 100 | Within 1 hour |
| 10 | 4 | 4 | 100 | Within 2 hours |
| 7.5 | 2 | 2 | 100 | Within a few hours |
| 5 | 10 | 5 | 50 | Within hours to 1 day |
| 2.55 | 2 | 0 | 0 | No mortality (7 days) |

BW: Body weight. Broiler chickens of both sexes were used for the lethality assessment, which was not stratified by sex or age. Group sizes differed between doses because the design was sequential. Additional chickens were tested only at doses with partial mortality.

#### *3.2.1. Clinical signs of profenofos*

Clinical signs were observed exclusively in broilers given the lower profenofos doses (2.55 and 5 mg/kg), as these birds survived long enough for signs to emerge. At doses of 7.5 mg/kg or higher, death occurred so swiftly (within minutes to hours) that no other clinical signs could be detected prior to collapse. In the surviving broiler chickens, profenofos exposure produced severe vomiting, severe defecation, and complete loss of appetite, together with neurological signs including tremors, weakness, and paralysis-like manifestations of the limbs. These surviving broiler chickens began to recover approximately 12 hours after the onset of signs, without any specific treatment.

### *3.3. Sulphur-based product*

Broiler chickens administered the sulphur-based product in feed at 300-2300 mg/kg BW demonstrated no immediate clinical signs, and no clinical signs or mortality were observed at 24 hours or at any point during the 7-day observation period. This group was therefore assessed based on its mortality and clinical signs (tolerance) endpoint. Serum biochemistry, intended as a secondary measure, could not be obtained for this group, and no biochemical data were reported. The complete absence of mortality and clinical signs up to the highest dose tested (2300 mg/kg BW) indicated that acute oral exposure to this sulphur-based product was well tolerated by broiler chickens under the conditions studied.

### *3.4. Effects of charcoal with L-carnitine*

Charcoal plus L-carnitine could only be evaluated in the broiler chickens that survived profenofos exposure, namely those given 2.55 and 5 mg/kg BW. Chickens at higher doses died too quickly for any dietary intervention to act. In these surviving broiler chickens, charcoal plus L-carnitine did not prevent feed refusal. The broiler chickens went off-feed for several hours after profenofos ingestion despite the additive. Because all surviving chickens received the additive and no profenofos-exposed group remained for comparison, it cannot be determined whether feed refusal occurred despite the additive or independently of it. This group was therefore reported as an exploratory observation rather than a test of protective efficacy.

## 4. Discussion

### *4.1. Emamectin benzoate*

In the present study, emamectin benzoate administered through drinking water at 300-2300 mg/kg BW produced a dose-related increase in serum ALP activity, whereas SGPT, SGOT, and serum creatinine remained largely unchanged, and no clinical signs or mortality were recorded. Serum ALP levels increased notably alongside normal skeletal growth during this period. However, normative values for broiler chickens demonstrated variability within this developmental stage, about 750 U/100 mL at 2 weeks versus 412 U/100 mL at 3 weeks[25]. This difference in ALP levels could not be attributed to emamectin benzoate and is likely due to age-related changes, so it is not considered a hepatobiliary effect. Serum creatinine demonstrated no evidence of kidney injury at the doses tested. These findings in broiler chickens contrasted with the marked toxicity reported for emamectin benzoate in other avian species[7,26]. Chukwudebe et al.[7] found emamectin benzoate to be highly toxic to the mallard duck (*Anas platyrhynchos*) and the Northern bobwhite quail (*Colinus virginianus*), and O'Grodnick et al.[26] reported subchronic and reproductive toxicity in the same two species, indicating pronounced species-specific sensitivity. In addition, Mobeen et al.[22] documented the toxicity of emamectin benzoate and its commercial formulations *in vivo* and *in vitro*, indicating that the formulated product can be toxic at doses well below those tolerated by the broiler chickens in the present study. Katagi and Fujisawa[18] noted wide interspecies variation in avian acute pesticide sensitivity, which was consistent with the present results and the marked toxicity reported in mallard duck and Northern bobwhite quail[7,26]. Broiler chickens exhibited only limited biochemical changes and no mortality, even at the highest tested dose of formulated emamectin benzoate (2300 mg/kg BW). The current results indicated that emamectin benzoate was strongly species-dependent and that broiler chickens were less acutely susceptible than the wild avian species.

### *4.2. Profenofos*

Profenofos, administered by direct oral gavage at 2.55-600 mg/kg BW, was the most acutely toxic of the three compounds, producing dose-related mortality with a Spearman-Kärber $LD_{50}$ of 4.7 mg/kg BW. Broiler chickens that received 7.5 mg/kg and higher doses died within minutes to a few hours, whereas surviving chickens at the lowest doses demonstrated cholinergic and neurological signs, vomiting, profuse defecation, loss of appetite, tremors, weakness, and paralysis-like limb signs, before recovering after about 12 hours. This high avian susceptibility was consistent with previous reports in chickens and other bird

species[1,28]. Aboul el Wafa et al.[1] reported that profenofos feeding altered serum biochemistry in chickens and adversely affected food consumption and BW. Similarly, Eglal et al.[28] reported reduced food consumption, impaired BW gain, and measurable tissue residues. The previous findings broadly aligned with the appetite loss and feed refusal observed in surviving chickens in the present study, although the previous studies used repeated dietary exposure rather than the single high-dose gavage used in the present study. In addition, Kafle et al.[17] reported cholinergic clinical signs, mortality, and hepatic and renal histopathological damage in broiler chickens given profenofos, and derived an avian oral $LD_{50}$ of about 16 mg/kg BW, although higher than the 4.7 mg/kg estimated in the present study. These values indicated high acute toxicity relative to typical avian $LD_{50}$ ranges[18]. Non-target avian toxicity has likewise been reported for wild birds such as the white egret[29], consistent with birds' high susceptibility to organophosphate exposure.

### *4.3. Sulphur-based product*

The absence of toxicity with the sulphur-based product was consistent with the well-established low acute oral toxicity of elemental sulphur, which was generally regarded as safe for mammals and birds and is widely used as an agricultural fungicide and acaricide[15]. The tolerance observed up to the highest tested dose was therefore anticipated rather than unusual, suggesting that acute oral exposure to this sulphur-based product does not cause obvious toxicity in broiler chickens under the studied conditions.

### *4.4. Mitigation strategies*

In the current study, the combination of dietary charcoal and L-carnitine was examined as an in-feed mitigation strategy against profenofos; however, it yielded no observable benefit. Its efficacy could only be assessed in broiler chickens that survived the lower doses of profenofos, and in these cases, it did not prevent post-ingestion feed refusal. This outcome was consistent with the recognized limitations of activated charcoal as an adsorbent. Charcoal binds numerous ingested toxins within the gastrointestinal tract and is most effective when administered promptly after ingestion, with its effectiveness influenced by the toxin's physicochemical properties[11,27]. The documented benefit of charcoal in poultry is primarily related to its preventive ability to adsorb dietary mycotoxins such as aflatoxin and deoxynivalenol, which are continually present in feed. Charcoal does not combat rapidly absorbed organophosphate boluses that lead to cholinergic collapse within minutes. In this study, profenofos was administered as a single gavage dose, acting before an in-feed additive could realistically adsorb it, which likely explains the lack of protection. L-carnitine, mainly studied in poultry for growth and metabolic support, was not expected to counteract acute cholinesterase inhibition[21,30]. These mitigation strategies in the present study indicated that in-feed charcoal combined with L-carnitine was ineffective for acute organophosphate poisoning, and that its failure reflected the exposure model used rather than a lack of adsorbent activity.

## 5. Conclusion

This preliminary study evaluated acute oral doses of emamectin benzoate, profenofos, and a sulphur-based compound in broiler chickens. Profenofos exhibited the highest acute toxicity, with an estimated oral $LD_{50}$ of 4.7 mg/kg BW and a 100% mortality rate at doses of 7.5 mg/kg or higher. Emamectin benzoate caused no mortality or clinical signs at doses up to 2300 mg/kg BW. The increase in serum ALP was likely due to age-related factors rather than liver toxicity. Chickens administered the sulphur-based product tolerated sulphur at all tested doses over a seven-day period. The present study had several limitations. First, a single control, sampled at 15 days, was compared with chickens sampled at 18 and 20 days, preventing distinction of the ALP increase from age-related growth. Second, the three products were administered via different, non-comparable routes, with emamectin benzoate and sulphur delivered at the pen level as unverified nominal doses. The study did not measure blood cholinesterase activity, which would have confirmed organophosphate-specific toxicity in the profenofos group due to setting constraints. Therefore, future studies should monitor and regulate pesticide residues in poultry feed, supported by coordinated action among regulators, farmers, feed manufacturers, and consumers, integrated pest management, and use of safer alternatives such as biopesticides.

## Declarations

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### *Authors' contributions*

Debashis Dutta performed the experimental procedures, analyzed the data, and contributed to manuscript preparation and drafting. Subhashish Batabyal, Apratim Maity, and Mayukh Ghosh provided research supervision, assisted with data interpretation, and revised the manuscript. All authors read and approved the final edition of the manuscript.

### *Availability of data and materials*

The data used and analyzed during this study are available from the corresponding author upon reasonable request.

### *Ethical considerations*

Plagiarism, consent to publish, misconduct, data fabrication and/or falsification, double publication and/or submission, and redundancy have been checked by all authors. The authors confirmed that they did not use AI-assisted text generation in preparing this study and the final manuscript. The authors declare and accept full responsibility for the originality of the article's content before submission and after acceptance.

### *Competing interests*

The authors declared no competing interests.

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